POSTNATAL MASCULINIZATION OF THE FEMALE RAT BY MEANS OF TESTOSTERONE PROPIONATE

HANS SELYE

Department of Anatomy, McGill University, Montreal, Canada

TWO PLATES (NINE FIGURES)

The fact that androgens have a masculinizing effect on female embryos both in the bird and in mammals has been proven beyond doubt by the numerous clear-cut experments of Kozelka and Gallagher ('34), Greene and Ivy ('37), Hamilton and Gardner ('37), Wolff ('38), Dantchakoff ('38 a, b, c), Willier, Rawles and Koch ('38), Raynaud ('38) and Greene, Burrill and Ivy ('38 a, b). In the bird, these authors injected the androgen directly into the developing ovum while in mammals it was administered to the mother during gestation. These experiments showed that under the influence of androgens, the derivatives of the wolffian duct develop in female embryos approximately in the same manner as they usually develop in males. The transformation of the female gonad itself into a testis-like structure, on the other hand, has never been clearly demonstrated in mammals although it has repeatedly been observed in the bird.

Greene and Burrill ('39) have shown furthermore, that postnatal treatment of young female rats with the luteinizing fraction of pregnancy urine may lead to excessive development of the clitoris, and Greene, Burrill and Ivy ('38 c) noted masculinization of the external genitalia of newborn female rats treated with testosterone propionate. In our previous experiments (Selye, '39), we observed that in adult mice, testosterone causes follicular maturation in the ovary. In our publication we did not discuss the response of the accessory genitalia but we might mention here that although

the clitoris showed marked enlargement, there was no evidence of masculinization of the internal genital organs; in fact, the uterus showed marked hyperemia and enlargement. This corresponds to the findings of previous workers. In view of these observations, it appeared of interest to establish whether the masculinizing effect which testosterone exerts on the internal genital organs is limited to the period of embryonic development or whether it may still be observed during the first weeks of postnatal life. Several earlier observations indicated that during the first few weeks of life, the responsiveness of the body to hormonal stimuli is not the same as at a later time. Thus in very immature rats, the luteinizing hormone of the urine of pregnant women fails to elicit follicle maturation and corpus luteum formation and causes only theca luteinization (Selye and Collip, '33). In this respect, the very immature rat responds in the same manner as the hypophysectomized animal. We noted, furthermore (Collip, Selye and Thomson, '33) that while rats weighing more than 50 gm. cease to grow immediately after hypophysectomy, younger rats may continue to grow in the absence of the hypophysis until they reach a weight of about 50 gm. It appears, therefore, that the very immature animal may grow in the absence of the hypophyseal growth hormone. In a more recent experimental series, we injected 1 mg. of estradiol daily into young rats weighing 25 gm. on the average and observed that they proceeded to grow quite rapidly until they reached a weight of about 100 gm. while the same treatment caused cessation of growth in rats weighing 100 gm. or more. It is conceivable that the failure of estradiol to inhibit growth in the very immature rat is due to the fact that at this age, growth is not dependent on the stimulating effect of pituitary growth hormone whose production is inhibited by estrogens. These findings lead us to consider whether the response of the female internal genital organs to testosterone changes abruptly at birth or whether a masculinizing effect—such as has been described in the embryo—

is still demonstrable during the first weeks of postnatal development.

In order to answer this question, we injected a litter of eleven newborn rats (five males, six females) with 1 mg. of testosterone propionate intraperitoneally daily for a period of 15 days. After this, the dose was raised to 3 mg. and the injections were administered subcutaneously for 15 more days. At the end of the 30-day treatment period, all experimental animals and a litter of ten control rats (two males, eight females) receiving the same amount of cholesterol for the same length of time were sacrificed and their organs fixed in Bouin's fluid for histological examination. We used cholesterol in our control series since it is chemically related to testosterone without possessing the androgenic actions of the latter. The reason for injecting our rats intraperitoneally during the first 2 weeks was that the oil in which the sterols were dissolved tended to escape through the needle prick in the tenden skin of the newborn rats if the injection was made directly under the skin.

At autopsy, the most striking change observed was that the ovary could not be detected by naked eye inspection in the testosterone-treated animals. The uterus was very thin and at its end, the oviduct was detectable, but the entire region of the oviduct had assumed a gelatinous translucent appearance. In order to investigate the fate of the gonad, the entire region of the oviduct was serially sectioned. Microscopical examination revealed that the wall of the oviduct was almost entirely transformed into a gelatinous mass, reminiscent to some extent of Wharton's jelly, the dermal layer in the estrous sex skin of monkeys or the stroma of endometrial moles as described by Selye, Harlow and McKeown ('35). The mesosalpinx showed a similar gelatinous transformation (fig. 1). The ovary itself was very atrophic, consisting only of a few primordial follicles, most of which contained degenerating ova. The connective tissue stroma of the ovary was very well developed and almost scar-like in appearance. While the cortical portion of the gonad was obviously ovarian in type,

the medulla contained numerous epithelial tubules (figs. 2 and 3), thus giving the gonads an appearance very similar to that of an ovotestis as illustrated in the publication of Willier, Rawles and Koch ('38) who produced such a structure in the genetically female chick embryo by testosterone administration Although these epithelial cords and tubules resemble early embryonic testis tissue, their cells are not sufficiently differentiated to allow a definite diagnosis. It is quite possible that they are merely ductuli efferentes which invade the medulla of the ovary. In any case, it is obvious that epithelial tubules—such as are normally observed in the male gonad only —arose in the medulla of the ovary under the influence of testosterone. These tubules were in contact with a rete in the hylum region of the ovary while near their origin in the medulla of the gland, their cells resembled those of the granulosa in partially degenerated follicles (fig. 4). In several cases, a well-formed epididymis and a vas deferens were seen in the vicinity of the oviduct (fig. 5).

The uterus was very atrophic, especially with regard to its muscular coat (figs. 6 and 7). Its epithelium was well developed, though in many cases, it contained round homogeneous eosinophilic inclusions whose nature we were unable to elucidate. They bear a curious resemblance to degenerated ova. This atrophy of the uterus is all the more surprising since in older females testosterone invariably produced pronounced uterine enlargement as mentioned above.

The vagina was atrophic and short and did not communicate with the outside. The clitoris was very large and penis-like in appearance.

In summary, one might say that the changes in the genital organs were similar to those described by Greene, Burrill and Ivy ('38 b) in rats treated with testosterone during embryonic life. However contrary to the findings of the above-mentioned investigators, the uteri were atrophic in the present series. It should be emphasized, furthermore, that judged by the descriptions given by various authors, the development of the large gelatinous masses in the region of the oviduct is not

observed if androgens are given during prenatal development nor does there seem to be such a marked reduction in the size of the ovary or an invasion of its medullary region by epithelial cords and tubules. It appears to be evident from our experiment that a marked degree of masculinization may occur in female rats treated with large doses of an androgen during postnatal life.

In the males, the genital organs, especially the seminal vesicles, prostate and penis were extremely well developed but this is not surprising since testosterone is known to exert such an action and consequently, in this respect, the response during the first weeks of life did not differ from the response of older animals. It is noteworthy, however, that while in adult animals, large doses of testosterone cause marked testicular atrophy, no decrease in testis size was observed in our treated animals in comparison with the controls.

Although not directly relevant to the main subject of this paper, it is perhaps worth mentioning that the adrenals showed marked cortical atrophy both in the male and in the female animals of the testosterone-treated series. The weight of the adrenals in the testosterone-treated group varied between 11 and 16 mg. while in the controls, it ranged between 16 to 27 mg. The fact that testosterone decreases the adrenal weight in females—in which these glands are normally larger than in males—has been reported by McEuen, Selye and Collip ('37 a, b) and Selye ('39) showed a similar decrease in female mice. In the present series, however, the testosterone-treated males had adrenals even smaller than normal males, so that the effect should not be regarded as a mere transformation of the female type of adrenal into the male type but as an actual atrophy. The histological changes underlying this atrophy were quite characteristic and differed significantly from those observed after hypophysectomy. While in the hypophysectomized rat, the atrophy of the adrenal cortex begins in the region of the reticularis, in the present series the reticularis was normal in appearance but the cells of the glomerulosa were atrophic and in fact, one

gained the impression that the entire glomerulosa region is substituted by a dense connective tissue scar (figs. 8 and 9). This difference between the adrenal cortex of the hypophysectomized and the testosterone-treated rat is of importance insofar as it indicates that the androgen does not act merely by inhibiting the adrenotrophic hormone production of the hypophysis.

SUMMARY

Experiments on the rat indicate that testosterone is not merely capable of directing the development of undifferentiated embryonic genital organs into the male type but can also transform the fully differentiated postnatal female genitals into the male direction. The ovaries of young rats treated with high doses of testosterone propionate become atrophic but numerous epithelial tubules develop in their medullary region.

It is of interest that the uterus, which shows considerable enlargement as a result of testosterone treatment in older rats, underwent atrophy in the young animals of this series.

The wall of the oviduct and parts of the mesosalpinx underwent transformation into a gelatinous mass.

The adrenals showed cortical atrophy due to marked involution of the glomerulosa.

It is concluded that during the first weeks of life, the response of the rat to testosterone is different from that observed at a later time.

ACKNOWLEDGMENTS

The expenses of this investigation were defrayed in part through a grant in aid received from the Schering Corporation of Bloomfield, N. J. The author is especially endebted to Drs. G. Stragnell and E. Schwenk of the above corporation for the testosterone propionate used in these experiments and to Messrs. K. Nielsen, H. Torunski and C. Rasmussen of this department for their untiring technical assistance which was instrumental for the completion of this work.

LITERATURE CITED

COLLIP, J. B., H. SELYE AND D. L. THOMSON 1933 Gonad-stimulating hormones in hypophysectomised animals. Nature (Lond.), vol. 131, p. 56.

DANTCHAKOFF, VÉRA 1938 a Réalisation du sexe a volonté par inductions hormonales. III. Inversions et déviations dans l'histogenese sexuelle chez l'embryon du poulet traité par l'hormone male. Bull. Biol. de la France et de la Belgique, T. 72, pp. 188–231.

———— 1938 b Sur les propriétés gonadotropes paradoxales de la testostérone. C. r. Soc. Biol., vol. 129, pp. 946–949.

———— 1938 c Gewebeplastizität, Hormone und Geschlecht. Erg. Physiol., Bd. 40, pp. 101–163.

GREENE, R. R., AND M. W. BURRILL 1939 Experimental intersexuality: Masculinization of female rats by postpartum treatment with anterior-pituitary-like hormone. Proc. Soc. exper. Biol. and Med., vol. 40, pp. 514–516.

GREENE, R. R., M. W. BURRILL AND A. C. IVY 1938 a Further effects of androgenic substance on sexual development in the female white rat. Proc. Soc. exper Biol. and Med., vol. 38, pp. 1–3.

———— 1938 b The experimental production of intersexuality in the female rat. Am. J. Obstet and Gynecol., vol. 36, pp. 1038–1046.

———— 1938 c Masculinization of female rats by postnatal administration of male sex hormone. Proc. Soc. exper. Biol. and Med., vol. 38, pp. 4–5.

GREENE, R. R., AND A. C. IVY 1937 The experimental production of intersexuality in the female rat with testosterone. Science, vol. 86, pp. 200–201.

HAMILTON, JAMES B., AND WILLIAM U. GARDNER 1937 Effects in female young born of pregnant rats injected with androgens. Proc. Soc. exper. Biol. and Med., vol. 37, pp. 570–572.

KOZELKA, A. W., AND T. F. GALLAGHER 1934 Effect of male hormone extracts, theelin, and theelol on the chick embryo. Proc. Soc. exper. Biol. and Med., vol. 31, pp. 1143–1144.

MCEUEN, C. S., H. SELYE AND J. B. COLLIP 1937 a Rôle of pituitary in effect of testosterone on the mammary gland. Proc. Soc. exper. Biol. and Med., vol. 36, pp. 213–215.

———— 1937 b Effect of testosterone on somatic growth. Proc. Soc. exper. Biol. and Med., vol. 36, pp. 390–394.

RAYNAUD, ALBERT 1938 Intersexualité obtenue expérimentalement chez la souris femelle par action hormonale. Bull. Biol. de la France et de la Belgique, T. 72, pp. 297–354.

SELYE, HANS 1939 Morphological changes in female mice receiving large doses of testosterone. J. Endocrinol., vol. 1, pp. 208–215.

SELYE, HANS, AND J. B. COLLIP 1933 Production of exclusively thecal luteinization and continuous oestrus with anterior-pituitary-like hormone. Proc. Soc. exper. Biol. and Med., vol. 30, pp. 647–649.

SELYE, HANS, C. HARLOW AND T. MCKEOWN 1935 The endometrial mole. Proc. Soc. exper. Biol. and Med., vol. 32, pp. 1253–1256.

WILLIER, B. H., MARY E. RAWLES AND F. C. KOCH 1938 Biological differences in the action of synthetic male hormones on the differentiation of sex in the chick embryo. Proc. Nat. Acad. Sci., vol. 24, pp. 176–182.

WOLFF, ETIENNE 1938 Sur l'existence d'hormones intermédiaires susceptibles de masculiniser les femelles et de féminiser les males chez l'embryon de poulet. C. r. Soc. Biol., T. 128, p. 420.

PLATE 1

EXPLANATION OF FIGURES

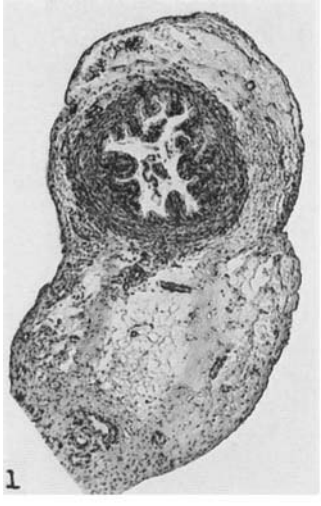
1 Oviduct of a testosterone-treated rat in cross section. Note gelatinous edema both in the wall of the oviduct and in the mesosalpinx.

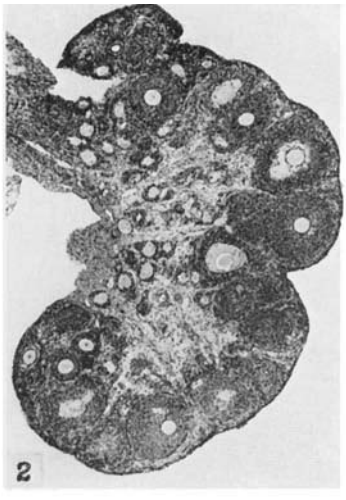
2 Section through the largest circumference of the normal control rat ovary. Although the follicles are immature, they are well developed and the connective tissue stroma is loose and contains no epithelial tubules.

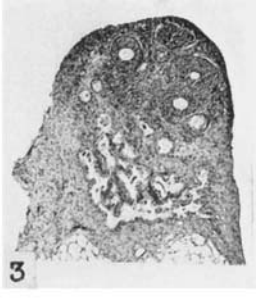
3 Section through the largest circumference of the ovary of a testosterone-treated rat. Note a great decrease in the total size of the gonad, the large number of degenerating follicles, the dense scar-like connective tissue and epithelial tubules in the hylum region.

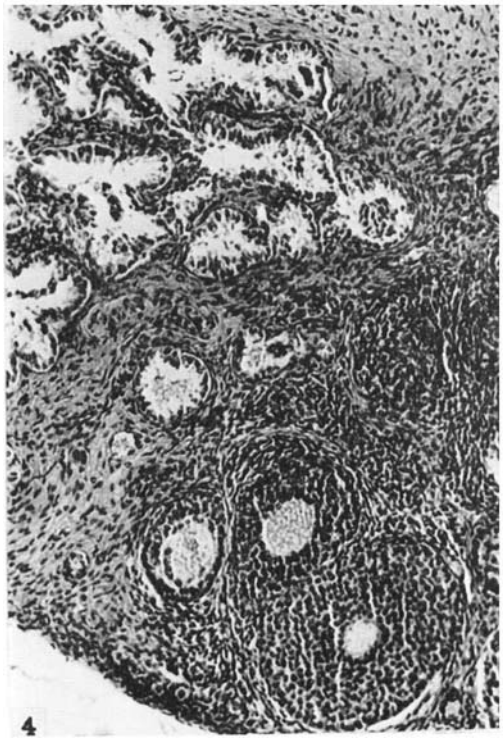
4 High magnification of the section in the medullary region of the ovary shown in figure 3. Note the similarity between the degenerating follicles and the epithelial tubules.

5 Epididymis and vas deferens in the parovarian connective tissue of a testosterone-treated female rat.


1


2


3


4

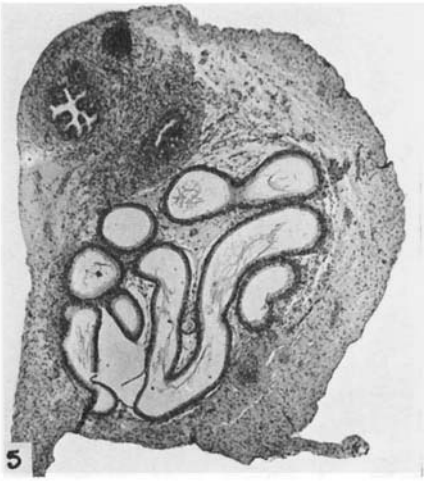

5

PLATE 2

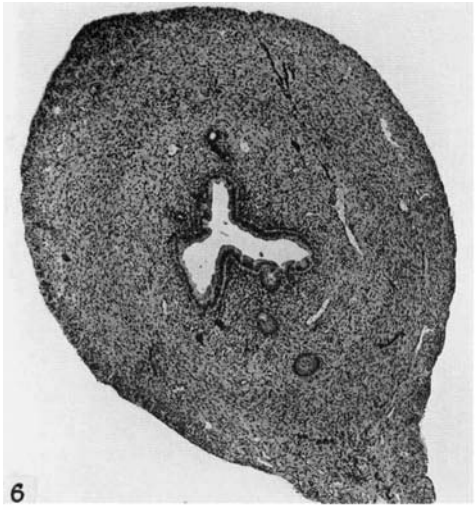
EXPLANATION OF FIGURES

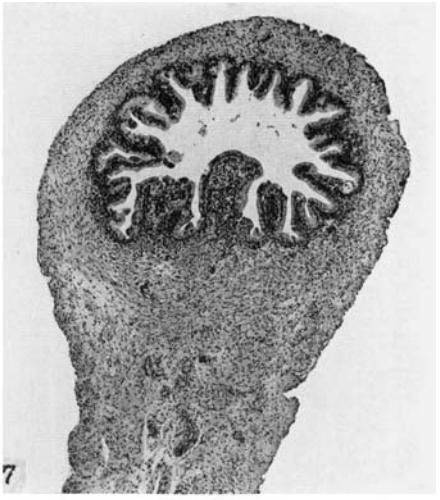
6 Cross section through the uterus of a normal control rat.

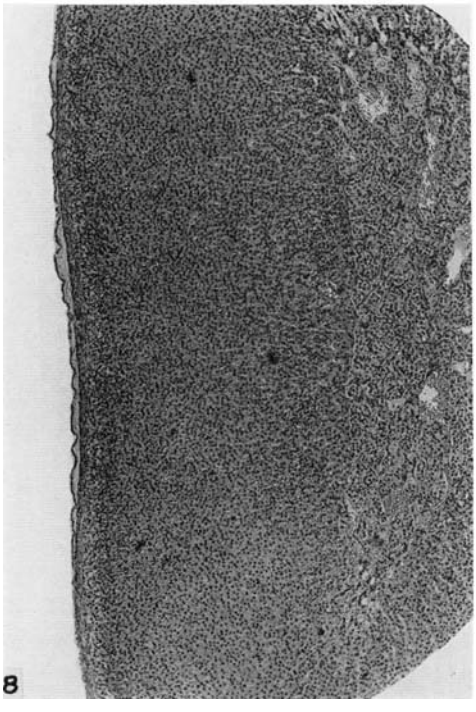
7 Cross section through the uterus of a testosterone treated rat. Note the atrophic muscular coat and the well-developed epithelial lining.

8 Section through the adrenal of a normal female control rat. The two adrenals in this animal weighed 21 mg.

9 Section through the adrenal of a testosterone-treated rat. Note the cortical atrophy and the dense scar-like connective tissue in the glomerulosa region. The medulla shows no significant change. The two adrenals of this animal weighed 14 mg.


6


7


8


9